夾竹桃紅顏料（Phloxine B）對東方果實蠅（*Bactrocera dorsalis*）之毒效評估

董耀仁　陳健忠*

台中縣霧峰鄉　行政院農業委員會農業試驗所應用動物組

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東方果實蠅（*Bactrocera dorsalis* (Hendel)），為亞太地區果樹之重要害蟲，成蟲產卵在果實內，幼蟲孵化後取食果肉，引起果實腐爛或落果，不但造成經濟損失，且因檢疫問題而阻礙鮮果外銷。由於其寄主植物多達百餘種，因此終年發生，不易防治[2]。

在果實蠅防治上，使用蛋白質水解物於點噴、大面積噴灑或放置誘蟲器內，馬拉松一直是主要的配合藥劑[15]。雖然馬拉松具有對哺乳類動物低毒與價格便宜之優點，但是為了防治果實蠅頻頻施用農藥，仍常引起消費者的疑慮與對天敵的傷害，使研究人員致力研發馬拉松的替代物[13]。夾竹桃紅顏料（Phloxine B；2',4',5',7'- tetrabromo-4,5,6,7-tetrachloro-,disodium salt），在美國已登記使用於藥物及化妝品作為添加顏料，使用數十年未有不良反應發生[6]。Phloxine B 屬光活性分子的一種，藉由光照後，對細胞產生毒性反應[3]，因此昆蟲食入含有 Phloxine B 的餌劑後經照光會死亡。因不具接觸毒和需食入且照光方能殺蟲的特性，其對人畜之毒性遠低於馬拉松，且在田間使用時，不像含馬拉松的餌劑有接觸性毒害。

果實蠅之腸道為半透明具有透光性，因此被認為適合使用此類光活性分子加以防治[14]。至今已有包括地中海果實蠅（*Ceratitis capitata* (Wiedemann)）、墨西哥果實蠅（*Anastrepha ludens* (Loew)）、西印度果實蠅（*Anastrepha obliqua* (Macquart)）等證實對 Phloxine B 具感受性，且田間試驗結果顯示 Phloxine B 具有成為田間防治用藥的潛力[5, 9, 11, 13, 16]。前人研究指出東方果實蠅雄蟲取食含有 4.29×10^{-2}M 以上濃度 Phloxine B 之甲基丁香油，於照光 2 小時內可達 100 %死亡率且濃度增加死亡率會提高[7]。於番石榴每週噴灑含有 Phloxine B 的酵母抽出

* 通訊作者。E-mail: chiencc@wufeng.tari.gov.tw

物，證明可有效壓制東方果實蠅及其他果實蠅族群(10)。但是噴灑含有 Phloxine B 的蛋白質水解物，易受下雨沖刷影響其防治效果，本試驗乃配製含 Phloxine B 之乾式餌劑，於室內測試其對東方果實蠅之毒效。

自霧峰番石榴園採得的果實蠅幼蟲，在室內以人工飼料飼育繁殖作爲供試蟲源。幼蟲培養基依邱(1) 之配方配製，成蟲則以酵母抽出物加蔗糖（1:3）配成之成蟲飼料飼養於飼育箱（30 x 30 x 30cm）內，飼養環境爲 25 ±1℃，光照 8 小時。供試藥劑爲 Phloxine B (C.I. no. 45410；Sigma-Aldrich Co.)，供試濃度爲將 Phloxine B 加入成蟲飼料中，配成 0.125 %、0.25 %、0.5 %、1 %、2 %及 4 % 六種不同濃度餌劑備用。

表一、東方果實蠅於無光環境下取食含有 Phloxine B 飼料經 4 小時後更換成正常飼料分別置於無光及光照 12 小時環境之死亡率

Table 1. Mortality of *Bactrocera dorsalis* fed phloxine B-containing diets in the dark for 4 h and then kept completely in the dark or in an environment with a photoperiod of 12: 12 (L: D) h

Conc. of phloxine B (%)	Mean percent mortality after feeding (± SE)[1]							
	Completely in the dark							
	1 h		4 h		8 h		24 h	
	Female	Male	Female	Male	Female	Male	Female	Male
0.125	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
0.25	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
0.5	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
1	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
2	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
4	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
Control	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
	With a photoperiod of 12: 12 (L: D) h							
	1 h		4 h		8 h		24 h	
	Female	Male	Female	Male	Female	Male	Female	Male
0.125	0.0 c	0.0 d	5.0 c (± 2.9)	5.0 c (± 2.9)	20.0 b (± 7.1)	7.5 c (± 4.8)	55.0 b (± 17.6)	12.5 bc (± 9.5)
0.25	5.0 c (± 2.9)	7.5 cd (± 7.5)	10.0 c (± 5.8)	7.5 c (± 7.5)	22.5 b (± 11.1)	10.0 c (± 10.0)	62.5 b (± 6.3)	32.5 b (± 14.9)
0.5	15.0 c (± 6.5)	15.0 c (± 2.9)	57.5 b (± 14.4)	67.5 b (± 2.5)	92.5 a (± 4.8)	87.5 a (± 6.3)	100.0 a	97.5 a (± 2.5)
1	75.0 b (± 11.9)	72.5 b (± 7.5)	100.0 a	90.0 a (± 0.0)	100.0 a	97.5 a (± 2.5)	100.0 a	100.0 a
2	97.5 a (± 2.5)	92.5 a (± 2.5)	100.0 a	97.5 a (± 2.5)	100.0 a	100.0 a	100.0 a	100.0 a
4	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Control	0.0 c	0.0 d	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c

[1] Means within a column followed by the same letter do not significantly differ at $p \leq 0.05$ (Fisher's protected LSD test; SAS Institute 1988).

測試毒效時將已先飢餓 12 小時之 16-18 日齡果實蠅成蟲，移入透明塑膠桶（直徑 15 cm x 高 20cm，上面覆蓋 100 目絹網）中，供給餌劑與水，並以未添加 Phloxine B 之成蟲飼料爲對照組，置於未開燈之定溫箱中（25℃)，經 4 小時充分取食後，進行下列三種試驗：(1)更換成正常之成蟲飼料，供給產卵器（白色底片盒，直徑 3 cm x 高 5cm，產卵器底部有 30 個直徑 0.1cm 圓孔，內塗番石榴汁誘引果實蠅產卵）並打開光源（Philips fluorescent lamp, TL 20W/54RS, 光照強度 8000 lux)，光照 8 小時後關燈，維持 12 小時光週期至第三天)。於光照期間紀錄取食後 1-8 及 24、48、72 小時之死亡蟲數，並於 1 天後記錄產卵數。每處理 20 對果實蠅，四重複。(2) 更換成正常之成蟲飼料，於持續黑暗環境下，紀錄取食後 1-8 及 24、48、72 小時之死亡蟲數，每處理 20 對果實蠅，四重複。(3)於黑暗中持續取食含 Phloxine B 餌劑，每天紀錄死亡蟲數至第七天爲止。每處理 20 對果實蠅，四重複。

死亡率及產卵數等試驗資料，以 SAS（Statistical Analysis System）[17]套裝統計軟體分析，並用 Fisher's protected least significant difference test (LSD) 比較各處理平均值之差異顯著性。

試驗結果顯示 Phloxine B 對東方果實蠅之殺蟲效果，明顯受光照影響。東方果實蠅取食含有 Phloxine B 之餌劑，4 小時後更換成不含 Phloxine B 之成蟲飼料於無光照的環境下，經 24 小時後處理組皆無果實蠅死亡（表一)，即使於後續觀察至第三天亦無果實蠅死亡（資料未列出)。而於有光的環境下，Phloxine B 對果實蠅的殺蟲效果，明顯受濃度影響，取食含有 4 % Phloxine B 的東方果實蠅雌、雄蟲於照光 1 小時達 100 %死亡率，而 2 %及 1 %處理組分別達 90 及 70 %以上的死亡率，均與對照組有顯著性差異。0.125、0.25 及 0.5 %三處理組於照光 1 小時無明顯的殺蟲效果，但 0.5 %處理組，於光照 8 小時後可達將近 90 %死亡率，24 小時後雌、雄蟲分別達 100 %及 97.5 %死亡率，與對照組間有顯著性差異（表一)。0.125、0.25 及 0.5 %處理組之死亡率於試驗後第 2 及第 3 天未再增加（資料未列出)。

於無光的環境下，Phloxine B 對東方果實蠅具有不同於光照時的殺蟲效果。於黑暗中連續取食含有 4 % Phloxine B 餌劑至第三天，對雌、雄蟲之致死率分別可達 97.5 及 87.5 %，2 %處理組則分別爲 91.3 及 81.3 %。1 %處理組到第六天才有較明顯的殺蟲效果，雌、雄蟲死亡率分別爲 82.5 及 68.8 %，而 0.125、0.25 及 0.5 %處理組則至第七天尚無良好之殺蟲效果（表二)。連續取食含有 Phloxine B 餌劑之東方果實蠅雌蟲，除 0.125 %處理組平均產 0.8 粒卵外，其餘處理組皆爲 0，顯著少於對照組之 186.5 粒卵（表三）。

Moreno and Mangan 以含有 Phloxine B 的蛋白質水解物置於養蟲箱中，上方提供能量強度 8 μmol $s^{-1}m^{-2}$ 螢光燈管照射，然後接入 20 對墨西哥果實蠅，經取食 24 小時後，將果實蠅移至室外，4 %處理組於光照 1 小時可達 98 %死亡率，而 1 %及 2 %處理組分別只有 37 及 49 %死亡率[12]。Liquido 等人於弱光約 2000Lux 的光照環境下，提供東方果實蠅雌蟲取食含有 1.36 % Phloxine B 之蛋白質水解物 4 小時後，換成正常飼料並將光照強度增加 5 倍，經 4 小時後，雌蟲死亡率可達 91 %[8]。本試驗之 1 % Phloxine B 處理組，於光照 1 小時，雌雄蟲死亡率分別爲

表二、東方果實蠅於無光環境下連續取食含有 Phloxine B 飼料之死亡率
Table 2. Mortality of *Bactrocera dorsalis* fed continuously on phloxine B-containing diets in the dark

Conc. of phloxine B (%)	Mean percent mortality after feeding (± SE)[1]							
	1 day		2 day		3 day		4 day	
	Female	Male	Female	Male	Female	Male	Female	Male
0.125	0.0 a	1.3 a (± 1.3)	0.0 c	1.3 a (± 1.3)	0.0 b	1.3 c (± 1.3)	0.0 c	2.5 bc (± 1.4)
0.25	0.0 a	0.0 a	0.0 c	0.0 a	0.0 b	1.3 c (± 1.3)	0.0 c	1.3 bc (± 1.3)
0.5	0.0 a	1.3 a (± 1.3)	0.0 c	1.3 a (± 1.3)	1.3 b (± 1.3)	1.3 c (± 1.3)	1.3 c (± 1.3)	1.3 bc (± 1.3)
1	0.0 a	0.0 a	0.0 c	2.5 a (± 1.4)	5.0 b (± 2.0)	2.5 c (± 1.4)	15.0 b (± 10.2)	3.8 b (± 1.3)
2	0.0 a	0.0 a	40.0 b (± 9.4)	27.5 a (± 6.3)	91.3 a (± 4.3)	81.3 b (± 1.3)	100.0 a	100.0 a
4	2.5 a (± 1.4)	0.0 a	62.5 a (± 1.4)	10.0 a (± 2.0)	97.5 a (± 2.5)	87.5 a (± 2.5)	100.0 a	100.0 a
Control	0.0 a	0.0 a	0.0 c	0.0 a	0.0 b	0.0 c	0.0 c	0.0 c

	5 day		6 day		7 day	
	Female	Male	Female	Male	Female	Male
0.125	0.0 c	2.5 bc (± 1.4)	0.0 c	2.5 c (± 1.4)	1.3 c (± 1.3)	2.5 c (± 1.4)
0.25	1.3 c (± 1.3)	2.5 bc (± 1.4)	1.3 c (± 1.3)	2.5 c (± 1.4)	1.3 c (± 1.3)	2.5 c (± 1.4)
0.5	2.5 c (± 1.4)	2.5 bc (± 1.4)	2.5 c (± 1.4)	2.5 c (± 1.4)	5.0 b (± 2.9)	13.8 b (± 3.1)
1	16.3 b (± 9.7)	5.0 b (± 2.0)	82.5 b (± 6.0)	68.8 b (± 3.8)	97.5 a (± 1.4)	97.5 a (± 1.4)
2	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
4	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Control	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c

[1] Means within a column followed by the same letter do not significantly differ at $p \leq 0.05$ (Fisher's protected LSD test; SAS Institute 1988).

75 及 72.5 %，4 %處理組更達 100 %死亡率（表一）。雖然目前一般防治果實蠅之食物餌劑多爲噴施以水稀釋之含毒蛋白質水解物，但本試驗之結果顯示使用含 Phloxine B 之乾式餌劑對東方果實蠅雌、雄蟲亦有良好之殺蟲效果。

與 Phloxine B 屬同類型顏料之玫瑰紅顏料（rose bengal）經研究對火蟻（*Solenopsis richteri* (Forel)）存在著不需光即可殺蟲之機制[4]，但此殺蟲機制明顯較需光照者弱。本試驗之結果顯示 Phloxine B 在無光環境下連續餵飼對東方果實蠅具有殺蟲效力（表二）。試驗過程中觀察到取食含有 0.125 %及 0.25 %

表三、東方果實蠅雌蟲取食含有 phloxine B 飼料對生殖力的影響

Table 3. Fecundity of female *Bactrocera dorsalis* after 4 h of feeding on phloxine B- containing diets

Conc. of phloxine B (%)	No. of eggs produced per female during 24 h (± SE)[1)]
0.125	0.8 (± 0.5) b
0.25	0.0 b
0.5	0.0 b
1	0.0 b
2	0.0 b
4	0.0 b
Control	186.5 (± 11.0) a

[1)] Means within a column followed by the same letter do not significantly differ at $p \leq 0.05$ (Fisher's protected LSD test; SAS Institute 1988).

Phloxine B 餌劑未死之果實蠅，其在取食含 Phloxine B 餌劑後呈現紅色之腹部，會隨著排出紅色的排泄物後而顏色慢慢恢復正常，顯示中毒而未死之果實蠅會將體內的 Phloxine B 排出體外。於黑暗中連續取食含有 Phloxine B 餌劑之果實蠅，在低濃度之 0.125、0.25 %處理組，連續取食七天後仍無顯著殺蟲效果，但隨著濃度提高果實蠅死亡時間提早。此可能爲低濃度處理組之 Phloxine B 被果實蠅排出體外，而高濃度處理組雖亦有部份被排出體外，但因蟲體內累積的劑量已達一定濃度而引發中毒死亡。Phloxine B 於黑暗環境下殺蟲機制是否爲濃度的累積所引發，仍需進一步試驗。

Liquido 等人於試驗過程中發現，取食含較高濃度 Phloxine B 蛋白質水解物時，東方果實蠅會有反芻現象，他們認爲此有助毒殺東方果實蠅族群中未取食到餌劑的果實蠅 [8]。但在本試驗光照處理組中死亡蟲數集中於前 24 小時，且 24 小時後低濃度處理組之果實蠅死亡率不會再增加 (資料未列出)，顯示 Phloxine B 的殺蟲效果雖然快速，但光照後蟲體內 Phloxine B 之殺蟲活性亦可能很快消退。反芻或排泄出的 Phloxine B 是否具有足夠的殺蟲效力，有待進一步證實。

取食含有 Phloxine B 餌劑的東方果實蠅雌蟲，會降低其產卵量 (表三)，其作用機制尚不明瞭，值得進一步研究。東方果實蠅取食餌劑後即使未死亡但卻不再產卵爲害，亦有助於提升 Phloxine B 於田間的應用性。

(關鍵詞：果實蠅、毒性、生殖力、毒餌、害蟲防治)

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ABSTRACT

Dong, Y. J., and Chen, C. C.* 2005. Evaluation on the toxic effects of phloxine B to the Oriental fruit fly (Diptera: Tephritidae). Plant Prot. Bull. 47: 69-76. (Agricultural Research Institute, Council of Agriculture, Executive Yuan, Wufeng, Taichung, Taiwan 413, ROC)

Tests were conducted to determine the toxic effects of phloxine B (a xanthene dye) to the Oriental fruit fly, *Bactrocera dorsalis* (Hendel). Dye-containing diets were made of 25 % yeast autolysate, 75 % sugar, and various concentrations of phloxine B (0.125 %, 0.25 %, 0.5 %, 1 %, 2 %, and 4 %). The tested adult flies were starved for 12 h prior to the experiment, and then allowed to feed on various diets for 4 h in the dark. After 4 h of feeding, these flies were separated into 3 groups with each group consisting of flies from all of the various groups fed different concentrations of dye. The 3 groups were then kept under the following conditions, respectively: (1) light first at an intensity of 8000 lux for 8 h, then dark, followed by a 12: 12 (L: D) h photoperiod throughout the remainder of the 72-h experimental period and fed a non-phloxine B diet; (2) complete darkness throughout the 72-h period and fed a non-phloxine B diet; (3) totally dark for 7 d with a phloxine B-containing diet. The results showed that in dye-fed flies kept under condition (1), 100 % mortality was observed after 1 h of exposure to the light in both sexes of flies fed previously with 4 % phloxine B. For the dye-fed flies kept completely in the dark and fed the non-dye food, no mortality occurred within 72 h. However, if the flies were kept completely in the dark but were fed the phloxine B diet, 100 % mortality occurred in the flies which had fed on 4 % phloxine B. These results indicate that phloxine B elicits light-independent toxicity in Oriental fruit flies; however, the light may have facilitated its toxic effects. In addition, female flies fed the dye laid fewer eggs. Our study suggests that phloxine B has the potential to be used as an environmentally friendly control agent for Oriental fruit flies, and further investigations should be carried out.

(Key words: fruit flies, toxicity, fecundity, poisoned bait, pest control)

*Corresponding author. E-mail: chiencc@wufeng.tari.gov.tw